van der Waals Host–Guest Complexes: Can One Predict Complexation Selectivity of Neutral Guests by a Cryptophane? MD-FEP Studies in Gas Phase and Chloroform Solution

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ABSTRACT: Quantitative assessment of the ''best fit'' between neutral molecules and the cavity of a ''rigid'' neutral receptor is a challenging task in supramolecular chemistry, drug design, and biology. We investigate this question by molecular dynamics and free-energy perturbation simulations performed on the macrocyclic ligand *cryptophane-E* (**L**) and its **L·S** complexes with three tetrahedral guests (**S** = CH_2Cl_2, $CHCl_3$, and CCl_4) in the gas phase and in chloroform solution. The van der Waals interactions are shown to play a crucial role in the calculated complexation selectivity. Calculations using Lennard–Jones 6-12 potentials and ''standard'' OPLS R^*_{Cl} and ε_{Cl} parameters for the Cl atoms of **S** lead to a preference for CCl_4, in contrast to the selectivities observed experimentally in solution ($CHCl_3 > CH_2Cl_2 > CCl_4$). Based on systematic investigations of the relative free energies of binding of $CHCl_3$/**S**, we derive a set of R^*_{Cl} and ε_{Cl} van der Waals parameters that account for experimental binding data. Although the complexes are of the van der Waals type, their electrostatic representation is also crucial for correct calculation of relative stabilities. Thus, the recognition of the ''best guest'' stems from a subtle balance of distance and time-dependent, cumulative noncovalent interactions between atoms of **S** and of **L**, which require an accurate representation. In

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addition, even in a weakly polar solvent, like chloroform, solvation effects are shown to modulate the recognition of the neutral substrates. © 1998 John Wiley & Sons, Inc. J Comput Chem 19: 820–832, 1998

Keywords: cryptophanes; molecular dynamics; free energy perturbation; preorganization; binding selectivity; solvent effects; molecular recognition

Introduction

Among molecular host molecules possessing large three-dimensional cavities (spherands, cavitands, speleands, clefts, carcerands[1–5]), cryptophanes[6] occupy a particular place because of their high binding affinity toward neutral or charged tetrahedral guests in water as well as in weakly polar solvents. These molecules consist of two cyclotriveratrylene (CTV) fragments connected by three bridging $(CH_2)_n$ chains. It has been found experimentally that *cryptophane-E* (hereafter noted **L**; Chart 1), which has three propylene bridges, forms in $(CD_2Cl)_2$ solution stable complexes with several $CH_mX_nY_{4-m-n}$ (X, Y = Hal, Me) guests, with a preference for the $CHCl_3$ molecule. Collet et al.[7] referred to these complexes as ''van der Waals molecules''[8]; that is, structurally well-defined assemblies of two neutral species noncovalently bound to each other, which may have lifetimes of the order of several seconds in solution.

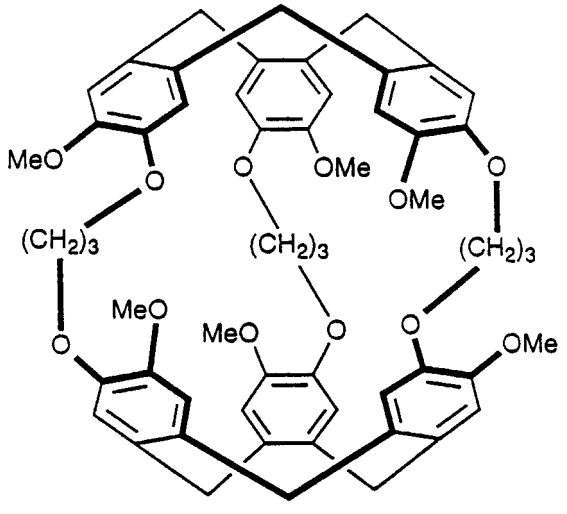


CHART 1. *Cryptophane-E.* (L)

The key question, which is the impetus of our study, concerns the steric/energetic factors that determine the ''best fit,'' and hence the binding selectivity. Based on the solid-state structure of the **L**-$CHCl_3$ complex[7] it has been estimated that the mean distance between the guest and arene carbons is about 20% larger than the sum of the van der Waals radii of contacting atoms.[9] Similar observations on the size of the cavity and the size of the ''optimal'' guest have been reported for *cryptophane-A*,[10] some cyclophanes,[11] and cyclodextrins.[12] This phenomenon was rationalized by Garel et al.[10] considering that the van der Waals forces between two neutral atoms are largest at a distance $r = 1.24 \cdot R_0$ (R_0 is the distance corresponding to the van der Waals energy minimum).[13] Such a suggestion, however, can be questioned, because the largest forces correspond to *the least* stable arrangement, whereas an energy minimum by definition corresponds to zero gradients (forces).

The validity of the ''best intrinsic fit'' concept has also been questioned on the basis of recent investigations in the field of host–guest complexes. As described recently by Schneider et al.[3] ''... deviations from the hole-size concept are more the rule than the exception.'' Based on an investigation of cyclodextrin complexes in water, he noted ''The popular view of supramolecular complexes—underscored by computer molecular modeling—generally is that a guest molecule will seek the closest contact, or fit, to the host cavity. Our results demonstrate that this view is too simplistic at least for cyclodextrins in water.''[12] Hence, the qualitative interpretation of cryptophane **L** complexation selectivity[6,7] requires further investigation. Does the order of the relative binding ability relate to the order of host–guest interaction energies and, in particular, does $CHCl_3$ display the most attractive interaction with **L**? To what extent is the macrocyclic cavity rigid and preorganized for complexation? Can it adjust to a given guest?

Another important question concerns solvent effects, which are known to modulate the com-

plexation selectivity of macrocyclic host molecules toward charged guests such as metal cations,[14,15] as well as toward neutral guests. For instance, based on simulations on the association of aromatic guests with a cyclophane in water, Jorgensen et al.[16] showed that the change in the hydration energy of the solution contributes to the host–guest binding affinities. Solvation energies have also been considered in the simulation studies of neutral guest complexes of hemicarcerands.[17] Experimentally, Smithrud and Diederich[18] observed a linear correlation between the free energies of formation of a cyclophane–pyrene complex and the solvent polarity parameter, $\mathbf{E_T(30)}$.[19] Schneider et al.[20] observed that association constants for the series of host–guest complexes of a fluorescent dye with macrocyclic hosts and analogues (tetraphenolate, α-cyclodextrin, and azoniacyclophane) are a function of the solvophobicity of the medium.

Although numerous experimental data on complexation of cryptophanes with neutral and charged guests have been published,[6,7,10,21–28] no detailed theoretical analysis of their complexation properties has been reported so far. The computational studies concern molecular mechanics calculations of the *cryptophane-A*·CH_4 complex.[10] FEP calculations on the chiral discrimination between CHFClBr complexes of *cryptophane-C*,[29] and MD simulations on the dynamics of the hexaacid derivative of **L** that is free[30] or complexed by TMA^+ in water.[31] The question of complexation selectivity has not been addressed computationally so far.

In this article, we report molecular dynamics (MD) and free-energy perturbation (FEP) simulations of *cryptophane-E* (**L**), uncomplexed and complexed with CH_2Cl_2, $CHCl_3$, and CCl_4 in the gas phase and in chloroform, with a particular focus on the relationships between the representation of the van der Waals interactions, solvation effects, and complexation properties of **L**. Chloroform was used as a solvent to mimic the $(CDCl_2)_2$ solvent used experimentally, for which no reliable force-field parameters are presently available. As the two solvents have similar $\mathbf{E_T(30)}$ polarity parameters [39.1 for $CHCl_3$ and 39.4 for $(CHCl_2)_2$],[32] it can be assumed that they also display similar solvation properties.

This article is organized as follows. We first report results obtained with ''standard'' OPLS Lennard–Jones 6-12 parameters, developed by fitting macroscopic properties of pure liquids,[33,34] but not thoroughly examined in host–guest complexes. We briefly describe structural features of the free and complexed ligand. The sequence of binding affinities, calculated with two different sets of charges, does not fit the experimental one for the $CCl_4/CHCl_3$ guests. We then analyze the possible reasons for such an inversion, and point out the representation of the Cl atoms of the guests. We describe FEP studies, performed systematically as a function of the charge distribution of **L** and **S**, and of the van der Waals parameters of **S**. The results are discussed in relation to experimental data and also in the context of the molecular recognition process in van der Waals (vdW) host–guest complexes.

Computation Procedure

Molecular dynamics calculations were performed with the AMBER4.1 program[35] with the following representation for the potential energy:

$$E = \sum_{\text{bonds}} K_r(r - r_{eq})^2 + \sum_{\text{angles}} K_\theta(\theta - \theta_{eq})^2 + \sum_{\text{dihedrals}} V_n(1 + \cos n\phi) + \sum_{i<j} \left(q_i q_j / R_{ij} - 2\varepsilon_{ij}(R^*_{ij}/R_{ij})^6 + \varepsilon_{ij}(R^*_{ij}/R_{ij})^{12} \right) \quad (1)$$

where vdW parameters R^*_{ij} and ε_{ij}, between unlike atoms i and j, were calculated using the commonly used Berthelot–Lorentz combination rules:

$$R^*_{ij} = R^*_i + R^*_j \quad (2)$$

$$\varepsilon_{ij} = (\varepsilon_i \varepsilon_j)^{1/2} \quad (3)$$

The parameters for most of the intramolecular and nonbonded intermolecular interactions came from the original AMBER force field.[36] Additional parameters concerning the CA—OS bonds and the CA—OS—CT, CA—CT—CT, and CA—CA—OS angles were taken from the AMBER* force field incorporated in the MacroModel program.[37] Torsional parameters for CA—CA—OS—CT dihedral angles were those of Grootenhuis and Kollman[38] (V_2 = 2 kcal/mol).

For the CH_2Cl_2, $CHCl_3$, and CCl_4 guest molecules we used an all-atom representation. The C—Cl bond length is not a standard AMBER parameter. The OPLS value of 1.767 Å[33] was somewhat larger than that found in the solid state for

the **L**·$CHCl_3$ complex (1.73 Å)[7], but close to the bond lengths of CH_2Cl_2, $CHCl_3$, or CCl_4, we obtained from *ab initio* (6-31G*) optimization at the SCF level using the SPARTAN program[39] (1.768, 1.763, and 1.767 Å, respectively), or those calculated at the MP2/6-31G* level[40] (1.767, 1.765, and 1.769 Å, respectively). The van der Waals parameters for C and H of the guests were taken, respectively, from the CT and HC atom types of AMBER (R^*_C = 1.80 Å, ε_C = 0.06 kcal/mol and R^*_H = 1.54 Å, ε_H = 0.01 kcal/mol).[36] They are similar to those used by Kovacs et al.[41] in the all-atom model of $CHCl_3$ (R^*_C = 1.79 Å, ε_C = 0.102 kcal/mol and R^*_H = 1.54 Å, ε_H = 0.0268 kcal/mol).

Two sets of atomic charges (see Chart 2) were used consistently for the host and the guest molecules: (i) charges generated by MacroModel,[37] and (ii) electrostatic potential (ESP) charges.[42] The ESP charges of the guests were calculated with SPARTAN[39] using the 6-31G* basis set. For $CHCl_3$, these charges correspond to a dipole moment of 1.49 Debye (larger than the experimental value of 1.01 Debye[43]). For the host molecule, the ESP 6-31G* charges, from Kirchhoff et al.,[30] were calculated on fragments of a hexaacid derivative of **L**. The charges for methyl groups were added to achieve the electroneutrality of the entire molecule.

For the $CHCl_3$ *solvent* molecules, we used a four-site model (where CH is represented in the united-atom approximation) with the OPLS parameters fitted on the bulk liquid properties of chloroform.[33]

The starting structures of the **L** free and of **L** complexed were derived from the solid-state structure of the **L**·$CHCl_3$ complex.[7] For the simulations in chloroform solution, the solute was placed at the center of a box of about 32–35 Å in length, containing 250–312 solvent molecules. The calculations were performed at constant volume using periodic boundary conditions. The residue-based cut-off for nonbonded interactions was set to 10 Å. After 1000 steps of conjugate-gradient minimization, the system was equilibrated for 5 ps of MD at 300 K starting with random velocities. This was followed by 250 ps of MD using a time-step of 1 fs. The temperature was maintained at 300 K by coupling to a thermal bath using a relaxation time of 0.1 ps. The 1–4 electrostatic and 1–4 van der Waals interactions have been divided by 8.0, such as in Kirchhoff et al.[30]

The differences in free energies between **L**·S_1 and **L**·S_2 host–guest complexes or between the uncomplexed S_1 and S_2 guests were calculated

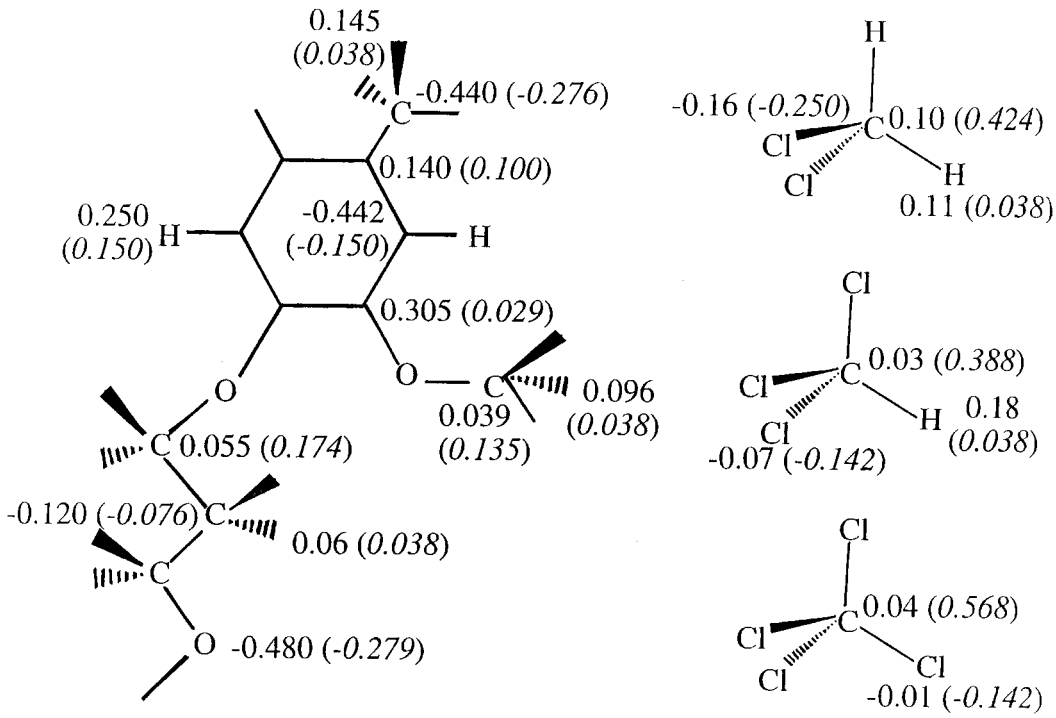


CHART 2. Charge distributions on **L** and guest complexes. ESP charges and charges from MacroModel (in parentheses).

using the statistical free-energy perturbation (FEP) theory[44] and the windowing technique, with:

$$\Delta G = \sum \Delta G_\lambda \text{ and}$$

$$\Delta G_\lambda = RT \cdot Log\langle exp(E_\lambda - E_{\lambda+\Delta\lambda})/RT\rangle\lambda$$

Formally, the $S_1 \rightarrow S_2$ mutation corresponds to a C—H → C—Cl bond transformation. The force-field parameters (**A**) of the mutated guest (force constant k_b, equilibrium bond length l_0, charges q_i, and van der Waals parameters R^* and ε) were calculated as a linear combination of the initial ($\lambda = 1$) and final ($\lambda = 0$) states: $\mathbf{A}(\lambda) = \lambda \cdot \mathbf{A}(S_1) + (1 - \lambda) \cdot \mathbf{A}(S_2)$. The system was first equilibrated for 250 ps. Each perturbation calculation consisted of 51 windows ($\Delta\lambda = 0.02$). Some calculations were performed with 101 windows ($\Delta\lambda = 0.01$). At each window 1000 steps of equilibration were followed by 4000 steps of data collection. All reported ΔGs were averaged from the forward ($\lambda = 1 \rightarrow 0$) and backward ($\lambda = 0 \rightarrow 1$) changes in free energies.

Additional test calculations were done in the gas phase with the MacroModel5.5 program (AMBER* force field)[37] using the windowing technique (11 windows) coupled with MD simulations. For the guest's atoms we used the same van der Waals parameters as in the AMBER4.1 calculations. At each λ, the energy of the system was minimized, and 10 ps of equilibration was followed by 250 ps of data collection.

The MD_DRAW program was used to visualize the structures and to analyze the structural results.[45] Because the **L** host molecule may be roughly described as a sphere, we calculated its effective size by the radius of gyration defined as:

$$R^2_{gyr} = \left(\sum r_i^2\right)/N_L$$

where r_i is the distance between the atom i and the center of mass of **L**, and the summation includes the N_L atoms of **L** (i.e., all atoms, except the O—Me groups).

Results

STRUCTURE AND DYNAMICS OF L AND ITS COMPLEXES IN GAS PHASE AND IN CHLOROFORM SOLUTION

Simulations on **L** *"uncomplexed."* In the gas phase, the simulations were started with the D_3 conformation of **L**, obtained from the x-ray structure of the **L**·$CHCl_3$ complex.[7] During the simulations with both ESP charges and with MacroModel charges, **L** lost its symmetry because of its tendency to fill its internal cavity with its own mobile groups. As illustrated in Figure 1, after 70 ps of simulation, the methylene fragment of one propylene chain moved toward the center of the cavity. Its two OC—CC torsional angles (*anti*, *anti*) differed from those of the two other chains, which remained (*gauche*, *gauche*), as in the x-ray structure.[7] Some methoxy groups rotated around the MeO—C_{ar} bond, and also "filled the cavity." Their distances to the center of mass of **L** varied from 1.7 Å (when one O—Me group points inward) to 7.8 Å. A similar behavior was observed in simulations with MacroModel charges.

In chloroform solution, simulations were performed with ESP charges. During the first 60 ps, the cavity of **L** remained empty. Then, one solvent $CHCl_3$ molecule became complexed and remained encapsulated in the cavity until the end of the simulation. This somewhat reduced the conformational freedom of the mobile parts of **L**, whose O—Me groups underwent fewer rotations. Moreover, none of the propylene chains could fill the cavity.

Simulations on the L·S Complexes (S = CH_2Cl_2, $CHCl_3$, CCl_4)

The calculations confirmed that, in its complexed state, *cryptophane-E* displays a relatively rigid cavity. Although the size of the guests in-

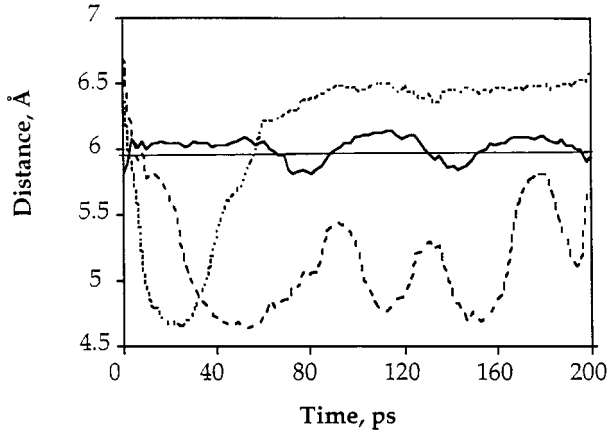


FIGURE 1. Distances between center of mass of **L** and the central carbon atoms of three bridging propylene fragments as a function of time (simulations performed with ESP charges). The horizontal line corresponds to the average value (5.9 Å) observed in the solid state for the **L**·$CHCl_3$ complex.[7]

creases in the series $CH_2Cl_2 < CHCl_3 < CCl_4$ (calculated volumes 58.0, 72.7, and 87.5 Å^3 with SYBIL-6.3[46]; 59.8, 74.4, and 88.6 Å^3 with MacroModel5.5[37]), the radius of gyration R_{gyr} remained nearly constant (5.22, 5.28, and 5.30 Å, respectively). These changes in R_{gyr} are small (0.06–0.08 Å) and of the same order of magnitude as the corresponding statistical fluctuations (0.10 Å).

In the three complexes, the guests rotated and changed their orientation with respect to the CTV fragments of **L**. For example, in the **L**·$CHCl_3$ complex, the guest molecule pointed its C—H bond toward either one CTV fragment, or toward one of the lateral chains (see Fig. 2, top). The lifetime of each ''orientation'' varied from 20 to 60 ps.

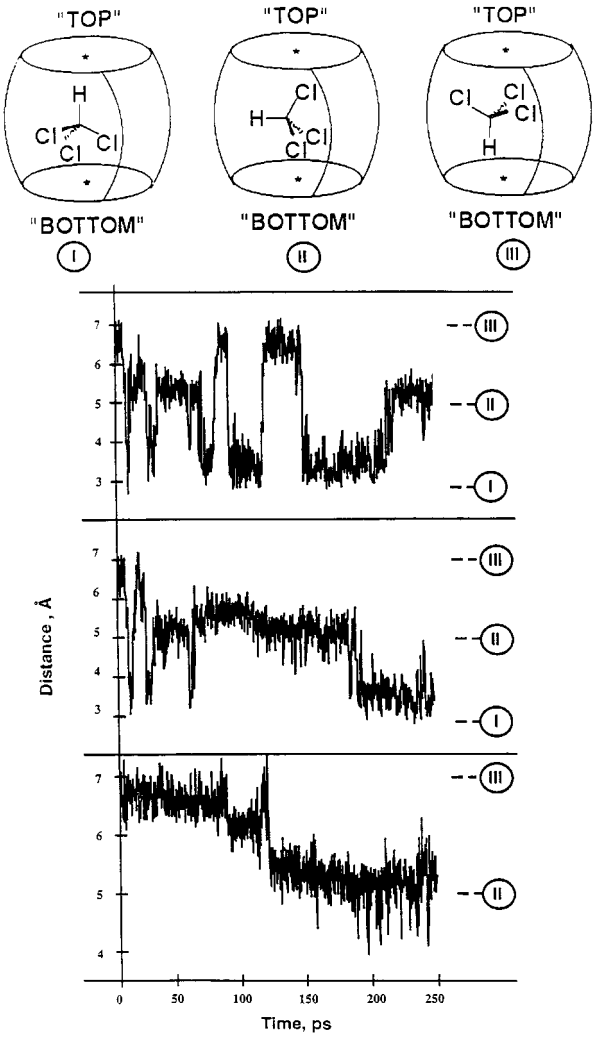
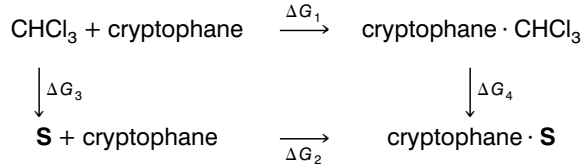


FIGURE 2. **L**·$CHCl_3$ complex. Distance between the H atom of the $CHCl_3$ guest molecule and center of mass of one CTV fragment of **L**, as a function of time. Calculation performed with ESP charges and R^*_{Cl} = 1.974 Å (top), 2.04 Å (middle), and 2.14 Å (bottom). The distances of 3 Å (**I**), 5 Å (**II**), and 7 Å (**III**) correspond to different orientations of C—H, as shown.

ANALYSIS OF L · S COMPLEXATION SELECTIVITY USING FEP CALCULATIONS

The thermodynamic cycle of Scheme 1 allows us to calculate the binding selectivity $\Delta\Delta G_c$ of **L** in solution for **S**/$CHCl_3$ guests. The selectivity, measured experimentally by $\Delta G_1 - \Delta G_2$, was calculated via the ''alchemical route'' as $\Delta\Delta G_c = \Delta G_3 - \Delta G_4$.[47] In this cycle, ΔG_4 corresponds to the differences of host–guest interactions in the **L·S**/**L**·$CHCl_3$ complexes, whereas ΔG_3 corresponds to the difference in solvation energies of the two uncomplexed guests. A negative value of $\Delta\Delta G_c$ corresponds to a preference of **L** for $CHCl_3$, compared with **S**:



SCHEME 1.

Free Energy Calculations with Standard OPLS van der Waals Parameters for Cl: Successes and Failures

It has been suggested than the peak of selectivity observed for $CHCl_3$ corresponds to an optimal fit between the cavity of **L** and this guest, compared with CH_2Cl_2, which is too small, and to CCl_4, which is somewhat too large.[7] Indeed, for the **L**·$CHCl_3$ → **L**·CH_2Cl_2 mutation, the ΔG_4 energy calculated *in vacuo* with ESP charges is positive (2.6 kcal/mol); that is, the **L**·$CHCl_3$ complex is intrinsically more stable than the **L**·CH_2Cl_2 one. In chloroform solution, ΔG_4 is similar to that in the gas phase (2.7 kcal/mol), and larger than ΔG_3 (1.6 kcal/mol). As a result, the difference in binding affinities in chloroform ($\Delta\Delta G_c = -1.1$ kcal/mol) favors $CHCl_3$, in close agreement with the experimental value (-0.9 kcal/mol) obtained in $(CDCl_2)_2$ solution[7] (Table I). Calculations with MacroModel charges also indicate a preference for $CHCl_3$ over CH_2Cl_2, with $\Delta\Delta G_c$ (-0.9 kcal/mol) in agreement with experiment.

In the case of the **L**·$CHCl_3$ → **L**·CCl_4 mutation, ΔG_4 is found to be negative in the gas phase as well as in chloroform solution (Table I), and ranges

from −0.9 to −2.0 kcal/mol, depending on the simulation conditions. Simulations performed in the gas phase with MacroModel, give nearly identical values to those obtained with AMBER4.1 (Table I), provided that the simulation time has been long enough. Calculations performed in chloroform result in ΔG_4 values similar to those obtained in the gas phase, indicating an *intrinsic preference of* **L** *for* CCl_4 (Table I). Taking into account the ΔG_4 and ΔG_3 energies calculated consistently in chloroform leads to values of $\Delta\Delta G_c$ (−0.4 kcal/mol with ESP charges and 0.2 kcal/mol with MacroModel charges) that are far different from the experimental results obtained in $(CDCl_2)_2$ solution ($\Delta\Delta G_c$ = −2.5 kcal/mol).[7] Next, the possible reasons for such a discrepancy are discussed.

On the Disagreement between the Calculated and Experimental CCl_4 / $CHCl_3$ Relative Binding Affinities: Crucial Role of van der Waals Interactions

Because the complexation experiments have been performed in $(CDCl_2)_2$ solution (these solvent molecules are too large to be complexed by **L**) and the calculations used chloroform as a solvent, the discrepancy could be due to marked differences in solvation effects. We feel this hypothesis to be unlikely, as the two solvents have similar polarity parameters $\mathbf{E_T(30)}$.[31,32]

On the computational side, the energy component analysis shows that the average $E_{L/S}$ interactions are attractive and increase with the size of **S** (−14.5, −17.5, −18.5 ± 1.5 kcal/mol, respectively, for CH_2Cl_2, $CHCl_3$, and CCl_4, with R^*_{Cl} = 1.947 Å and ESP charges), mostly due to the van der Waals contribution (from 88% to 90%). The importance of this contribution for discrimination of CCl_4 from $CHCl_3$ is also illustrated by the $\mathbf{L}{\cdot}CHCl_3 \rightarrow \mathbf{L}{\cdot}CCl_4$ mutation performed with electrostatic/van der Waals decoupling (Fig. 3) where $\Delta G_{4,\,vdW}$ decreases similar to ΔG_4, whereas $\Delta G_{4,\,elec}$ increases somewhat, as the dipole moment of the guest drops from 1.4 Debye to zero.

In the $\mathbf{L}{\cdot}CCl_4$ complex, the distances between any Cl atom of CCl_4 and its neighboring aromatic carbons (C_{ar}) are 3.2–4.0 Å. As shown in Figure 4 the van der Waals interaction between a pair of $C_{ar} \cdots Cl$ atoms is repulsive below 3.37 Å, and is most attractive at 3.797 Å. An increase of R^*_{Cl} shifts the minimum to larger distances and decreases markedly the repulsion in the ''critical'' range of $Cl \cdots C_{ar}$ distances of 3.2–4.0 Å (Fig. 4).

TABLE I.
Relative Free Energies (kcal / mol) of Mutation of $CHCl_3$ (Uncomplexed and Complexed by L) to S (S = CH_2Cl_2, CCl_4) in Gas Phase and in Chloroform. Calculations with OPLS van der Waals Parameters for Cl.[a]

	$CHCl_3 \rightarrow CH_2Cl_2$		$CHCl_3 \rightarrow CCl_4$	
Charges on **L** and **S**	ESP	MacroModel	ESP	MacroModel
ΔG_4 (**S** complexed)				
in vacuo	2.64 ± 0.02	1.62 ± 0.01	−1.24 ± 0.04	−2.02 ± 0.01
				−1.86 ± 0.11[e]
In chloroform	2.73 ± 0.03[f]		−1.04 ± 0.01[f]	
	2.70 ± 0.01	2.32 ± 0.01	−0.93 ± 0.01	−1.61 ± 0.03
ΔG_3 (**S** free)				
In chloroform	1.58 ± 0.01	1.40 ± 0.07	−1.41 ± 0.01	−1.40 ± 0.05
$\Delta\Delta G_c$				
Calcul.[b]	−1.06	−0.22	−0.17	0.62
				0.46[e]
Calcul.[c]	−1.15[f]		−0.48[f]	
	−1.12	−0.92	−0.37	0.21
exp.[d]	−0.9		−2.5	

[a] Unless otherwise specified, mutations were performed using the ''windowing'' technique (51 windows, 255 ps), dividing the 1–4 nonbonded interactions by 8.0, using a cut-off distance of 10 Å and OPLS parameters for Cl (R^* = 1.974 Å, ε = 0.30 kcal / mol). ''±'' is the difference between forward and backward mutations.
[b] Calculated as $\Delta\Delta G_c = \Delta G_{3,\,chl} - \Delta G_{4,\,gas}$.
[c] Calculated as $\Delta\Delta G_c = \Delta G_{3,\,chl} - \Delta G_{4,\,chl}$.
[d] Experimental differences in free energies of complexation in $(CDCl_2)_2$ solution (from Canceill et al.[7]).
[e] Calculated with the MacroModel5.5 program using the incorporated AMBER* force field.[37]
[f] Mutations performed using 21 windows, 105 ps.

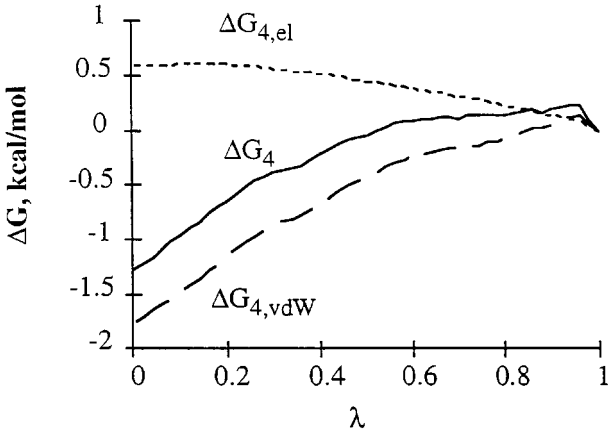


FIGURE 3. Mutation **L**·$CHCl_3$ → **L**·CCl_4 in the gas phase. Change in free energy (ΔG_4) and in its electrostatic ($\Delta G_{4,el}$) and van der Waals ($\Delta G_{4,vdW}$) components, as a function of λ (101 windows, 505 ps). The initial state (**L**·$CHCl_3$) corresponds to $\lambda = 1$. Calculations performed with ESP charges, R^*_{Cl} = 1.974 Å and ε_{Cl} = 0.30 kcal / mol.

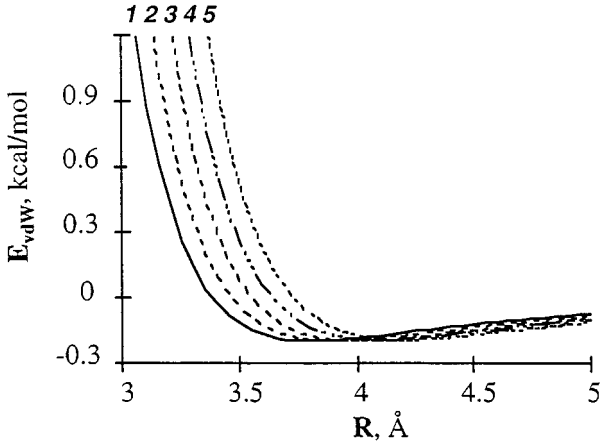


FIGURE 4. $C_{ar} \cdots Cl$ van der Waals interaction energy (kcal / mol), as a function of the interatomic distance (R, Å) calculated with R^*_{Cl} = 1.974 Å (1), 2.04 Å (2), 2.14 Å (3), 2.24 Å (4), and 2.34 Å (5), (R^*_C = 1.80 Å, ε_C = 0.06, and ε_{Cl} = 0.30 kcal / mol).

This analysis thus suggests that small modifications of the van der Waals parameters of the guest could reverse the $CHCl_3/CCl_4$ affinity. This is why we decided to vary R^*_{Cl} and ε_{Cl}, to tentatively fit the binding sequence by **L**. Because of the rotational motions of the guest inside the cavity, and of the large fluctuations of the host's energy, neither the host–guest interaction energy $E_{L/S}$ obtained from simple mechanics minimizations, nor the average $\langle E_{L/S} \rangle$ energy calculated from MD trajectories could be used to assess the relative binding preferences. Thus, it became clear that the force-field parameters had to be tested on FEP calculations, performed on time-scales long enough to sample the different states of the complexes. The results are discussed in what follows.

Calculation of Relative Binding Affinities as a Function of the van der Waals Parameters of Cl

We calculated relative free energies of binding as a function of R^*_{Cl} and ε_{Cl} parameters, to tentatively reproduce the relative experimental free energies of complexation ($CHCl_3 > CH_2Cl_2 > CCl_4$). Most of the calculations of ΔG_4 were performed in the gas phase. R^*_{Cl} was varied from 1.947 (OPLS value) to 2.34 Å. For ε_{Cl}, the values of 0.30 (OPLS parameter) and of 0.15 kcal/mol were compared. The two sets of charges (ESP/MacroModel) were also compared. Additional tests were performed in chloroform using selected parameters.

Calculations of ΔG_4. The ΔG_4 changes in free energies (Fig. 5) upon the **L**·$CHCl_3$ → **L**·**S** mutations (where **S** = CCl_4 or CH_2Cl_2) are found to depend markedly on the choice of parameters, including the atomic charges. In particular, with **S** = CCl_4, ΔG_4 is negative with MacroModel charges, but positive with ESP charges (ε_{Cl} = 0.30 kcal/mol) for most values of R^*_{Cl}. As expected, increasing R^*_{Cl} generally shifts ΔG_4 to larger values for **S** = CCl_4, and to smaller values when **S** = CH_2Cl_2, but the changes depend on the electrostatic representation of the system, as well as on the choice of ε_{Cl}. When ε_{Cl} is increased from 0.15 to 0.30 kcal/mol (Fig. 6), ΔG_4 increases when **S** = CH_2Cl_2 and decreases when **S** = CCl_4.

Calculations of ΔG_3. As expected, for the uncomplexed guests, **S**, the relative free energies of solvation, ΔG_3 are found to display a weak dependence on the R^*_{Cl} parameter and on the atomic charges on **S** (Fig. 7). In all cases, ΔG_3 becomes more attractive in the order $CH_2Cl_2 < CHCl_3 < CCl_4$. We also note that the differences are largest with the largest value of ε_{Cl} (0.30 kcal/mol).

Calculations of $\Delta\Delta G_c$. As a first approximation, we estimated the relative free energies of complexation, assuming that $\Delta G_{4,chl}$ (i.e., obtained in chloroform solution) is close to $\Delta G_{4,gas}$ and that $\Delta\Delta G_c = \Delta G_{3,chl} - \Delta G_{4,gas}$. Both terms were computed consistently. The results (Fig. 8) show that $\Delta\Delta G_c$ depends markedly on the choice of both electro-

TABLE II.
Relative Free Energies (kcal / mol) of Mutation of $CHCl_3$ (Uncomplexed and Complexed by L) to S (S = CH_2Cl_2, CCl_4) in Gas Phase and in Chloroform. Calculations with Modified van der Waals Parameters for Cl.[a]

	$CHCl_3 \rightarrow CH_2Cl_2$		$CHCl_3 \rightarrow CCl_4$	
R^*_{Cl}	2.04 Å	2.14 Å	2.04 Å	2.14 Å
ΔG_4 (**S** complexed)				
In vacuo	2.27 ± 0.02	2.42 ± 0.01	0.43 ± 0.05	1.06 ± 0.01
In chloroform	4.18 ± 0.01[e]	3.05 ± 0.03[e]	−0.27 ± 0.06[e]	0.82 ± 0.01[e]
	2.70 ± 0.01	2.70 ± 0.01	−0.51 ± 0.02	0.14 ± 0.01
ΔG_3 (**S** free)				
In chloroform	1.77 ± 0.02	1.94 ± 0.02	−1.53 ± 0.01	−1.67 ± 0.03
$\Delta\Delta G_c$				
Calcul.[b]	−0.50	−0.48	−1.96	−2.73
Calcul.[c]	−3.55[e]	−1.11[e]	−1.26[e]	−2.49[e]
	−0.93	−0.76	−1.02	−1.81
Exp.[d]	−0.9		−2.5	

[a]Unless otherwise specified, mutations were performed using the ''windowing'' technique (51 windows, 255 ps), dividing the 1–4 nonbonded interactions by (8.0), using a cut-off distance of 10 Å. ''±'' is the difference between forward and backward mutations.
[b]Calculated as $\Delta\Delta G_c = \Delta G_{3,chl} - \Delta G_{4,gas}$.
[c]Calculated as $\Delta\Delta G_c = \Delta G_{3,chl} - \Delta G_{4,chl}$.
[d]Experimental differences in free energies of complexation in $(CDCl_2)_2$ solution (from Canceill et al.[7]).
[e]Mutations performed using 21 windows, 105 ps.

static and of vdW parameters. Some of them do not fit the experimental data, others reproduce the selectivity for a couple of guests only, whereas others fit the right sequence for the experimental stabilities. Both $\mathbf{L}{\cdot}CHCl_3 \rightarrow \mathbf{L}{\cdot}CH_2Cl_2$ and $\mathbf{L}{\cdot}CHCl_3 \rightarrow \mathbf{L}{\cdot}CCl_4$ mutations *simultaneously* fit the experiment only if ESP charges, $R^*_{Cl} = 2.14$ Å and $\varepsilon_{Cl} = 0.30$ kcal/mol, are used. Calculations of $\Delta\Delta G_c$ using $\Delta G_{4,chl}$ (computed in chloroform solution) instead of $\Delta G_{4,gas}$ with these parameters give similar results (Table II and Fig. 9). It is thus possible to find a set of parameters that accounts for the relative free energies of binding in solution, using a Lennard–Jones representation of van der Waals interactions in conjunction with standard combination rules (Fig. 9). With these parameters, *the discrimination of the guests results from a subtle balance between short-range, weakly repulsive interactions and cumulative, attractive ones* (Fig. 4).

Discussion

DIFFICULTY IN ASSESSMENT OF VAN DER WAALS INTERACTIONS BETWEEN UNLIKE ATOMS

Efforts to reproduce experimental relative free energies require an accurate representation of the potential energy and, in the case of ''van der Waals complexes,'' of the nonbonded interactions. A first question concerns the analytical form used for such calculations: Can Lennard–Jones ''6-12'' potentials used with the standard combination rules (2)–(3) provide reasonable results? Some recent publications have addressed the question. Halgren[48] and Waldman and Hagler[49] have shown that both 6-12 potentials and Berthelot–Lorentz combination rules fail to precisely reproduce potential curves of two interacting rare-gas atoms. Chipot et al.[50] also found that application of these combination rules leads to unsatisfactory solvation energies (ΔG_{solv}) of halosubstituted alkanes they fitted separately in hexane solution. To reproduce ΔG_{solv}, van der Waals parameters A_{ij} and B_{ij} for Cl/CH_2 and Cl/CH_3 interactions. In another study on the solubility of water in a membrane, it was also suggested that specific A_{ij} and B_{ij} terms should be used for unlike atoms, instead of R^* and ε,[51] to reproduce the experimental surface tension between water and decane.[52]

In our study, agreement with related experimental data has been achieved using an all-atom model for $CHCl_3$ as guest, and a four-site model for $CHCl_3$ as solvent, with different charges and vdW parameters. The parameters obtained for the guests ($R^*_{Cl} = 2.14$ Å, $\varepsilon_{Cl} = 0.30$ kcal/mol) are close to those of Chipot et al.[50] ($R^*_{Cl} = 2.23$ Å, $\varepsilon_{Cl} = 0.312$ kcal/mol for C1/C2 interactions and

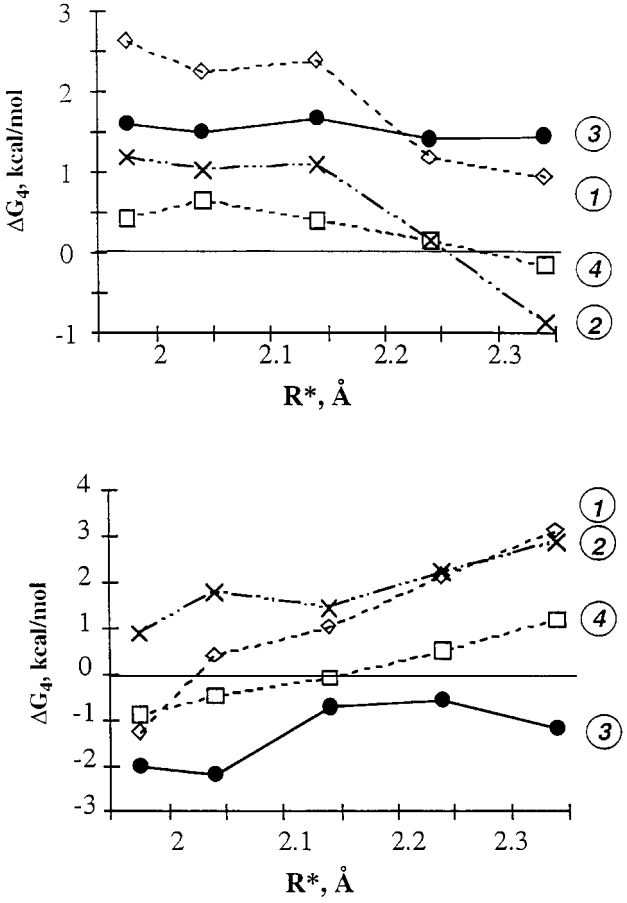


FIGURE 5. Mutation **L**·$CHCl_3$ → **L**·**S** in the gas phase. Changes in ΔG_4 as a function of R^*_{Cl} for **S** = CH_2Cl_2 (top), and **S** = CCl_4 (bottom). Calculations performed: with ESP charges and ε_{Cl} = 0.30 kcal / mol (curve *1*); with ESP charges and ε_{Cl} = 0.15 kcal / mol (curve *2*); with MacroModel charges and ε_{Cl} = 0.30 kcal / mol (curve *3*); and with MacroModel charges and ε_{Cl} = 0.15 kcal / mol (curve *4*). The positive values of ΔG_4 correspond to an intrinsic preference of **L** for $CHCl_3$ over **S**, whereas for negative values, **S** is preferred.

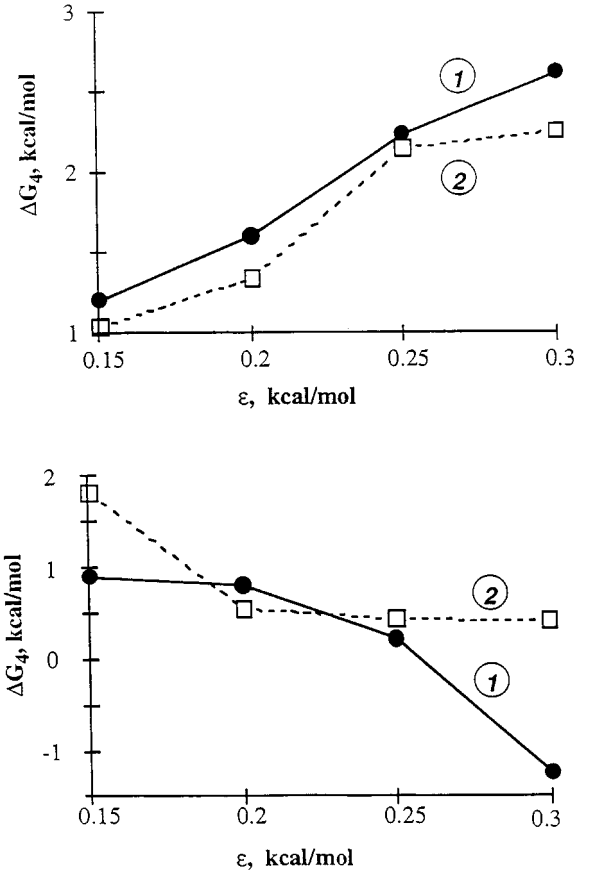


FIGURE 6. Mutation **L**·$CHCl_3$ → **L**·**S** in the gas phase. Changes in ΔG_4 as a function of ε_{Cl} for **S** = CH_2Cl_2 (top), and **S** = CCl_4 (bottom). Calculations with ESP charges and R^*_{Cl} = 1.974 Å (curve *1*), and R^*_{Cl} = 2.04 Å (curve *2*).

R^*_{Cl} = 2.16 Å, ε_{Cl} = 0.292 kcal/mol for C1/C3 interactions, where C2 and C3 are united atoms approximating CH_2 and CH_3 groups, respectively). The latter were calculated from the corresponding A_{ij} and B_{ij} parameters, using the Berthelot–Lorentz combination rules (2)–(3) and OPLS parameters for united atoms C2 and C3.

Regarding the difference between vdW parameters for $CHCl_3$ as a "guest" versus as a "solvent," we notice that the latter was fitted to reproduce characteristics of the pure liquid, where intermolecular interactions result mostly from mutual Cl ⋯ Cl interactions. They were not tested in host–guest systems. Indirect assessment of differ-

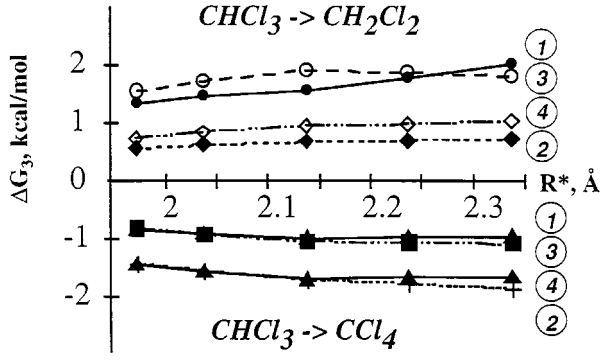


FIGURE 7. Mutation of uncomplexed guests $CHCl_3$ → **S** (**S** = CH_2Cl_2, CCl_4) in chloroform solution. Free energy (ΔG_3) as a function of R^*_{Cl}. Positive values correspond to the $CHCl_3$ → CH_2Cl_2 mutation, negative values correspond to $CHCl_3$ → CCl_4 mutation. See also Figure 5 for definitions of curves *1* to *4*.

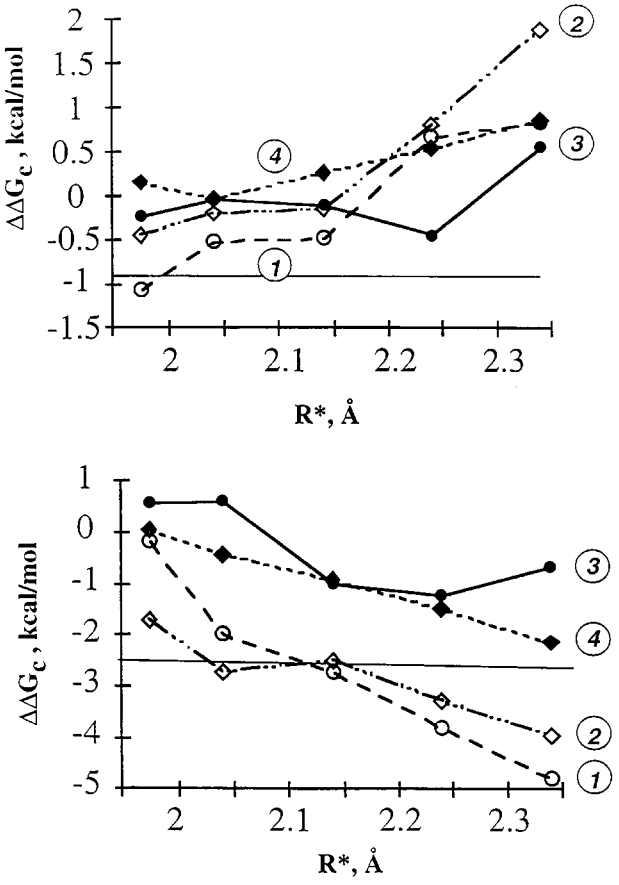


FIGURE 8. Mutation **L**·$CHCl_3$ → **L**·**S**. Differences in free energies of complexation ($\Delta\Delta G_C = \Delta G_{3,chl} - \Delta G_{4,gas}$) as a function of R^*_{Cl} for **S** = CH_2Cl_2 (top) and CCl_4 (bottom). See Figure 5 for definitions. The horizontal lines correspond to the experimental values.

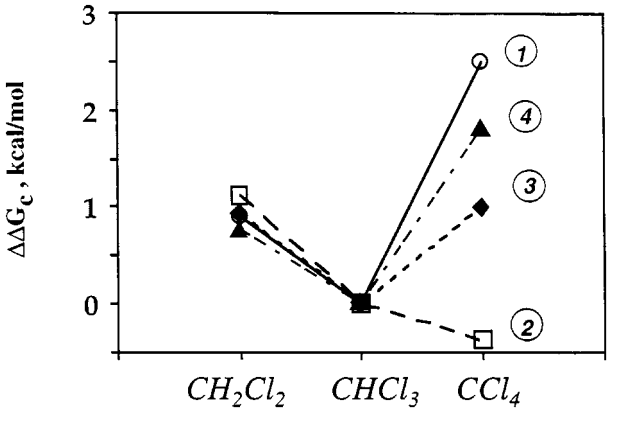


FIGURE 9. Relative free energies of complexation ($\Delta\Delta G_c = \Delta G_{3,chl} - \Delta G_{4,chl}$) of **L** with CH_2Cl_2, $CHCl_3$, and CCl_4 obtained experimentally (curve *1*) and calculated using ESP charges and ε_{Cl} = 0.30 kcal / mol and R^*_{Cl} = 1.974 Å (*2*), 2.04 Å (*3*), and 2.14 Å (*4*).

ences between these parameters comes from the statistical analysis of intermolecular contacts in the solid state. Based on x-ray diffraction data, Zefirov and Zorkii[53,54] showed that the vdW radius of Cl suggested by Bondi[9] (1.75 Å) was underestimated and should be increased to 1.90 Å. The main difference between the two approaches is that Zefirov and Zorkii overlooked structures involving Cl ··· Cl contacts from their statistical analysis, considering them as ''shortened intermolecular contacts,'' whereas Bondi used all available data. Using Zefirov's value of 1.90 Å, and taking into account that R^* is usually about 0.2 Å larger than the vdW contact radii derived from crystal packing data,[55] leads to $R^* \approx 2.10$ Å, which is very close to the value obtained in our study.

SOLVENT EFFECTS ON CCl_4 / $CHCl_3$ COMPLEXATION SELECTIVITY

Our simulations show that solvation effects contribute to the complexation selectivity, even in the case of neutral guests in weakly polar solvents. According to Scheme 1, the complexation selectivity in solution, measured by $\Delta\Delta G_c$, is a function of host–guest interactions and of the solvation of the **L** · **S** complex (via ΔG_4), and of the guest's desolvation energies (via ΔG_3). Because the guest, **S**, encapsulated in the cavity of cryptophane, is shielded from solvent, most of the solvent effect arises from the desolvation energy ΔG_3, which *modulates* the complexation selectivity. We calculate that ΔG_3 has the same order of magnitude as ΔG_4. In the case of the $CHCl_3 \rightarrow CH_2Cl_2$ mutation, both ΔG_3 and ΔG_4 contributions are positive. They partially compensate each other, which reduces the binding selectivity in chloroform solution compared with the gas phase. On the other hand, for the $CHCl_3 \rightarrow CCl_4$ mutation, ΔG_3 and ΔG_4 have opposite signs (i.e., the solvent enhances the discrimination between the two guests by **L**).

Solvent effects can be extremely important when two guests display similar interactions with the ligand, but differ in their solvation properties. This is likely the case for the smaller analogue of **L**, *cryptophane-A*, which forms tight complexes with CH_2Cl_2, but not with $CHClF_2$, which has a similar size.[10] This ''abnormal'' behavior was related by Garel et al.[10] to the higher solubility of fluorinated methanes in tetrachloroethane—that is, *by changes in solvation energies*. Related estimations of these data can be found in Luque et al.[56]

INTERNAL ROTATION OF GUEST IN CAVITY AND COMPLEXATION SELECTIVITY

Upon complexation, a molecular guest loses part of its translational and rotational freedom. The free-energy contribution caused by this entropy loss (''cratic'' free energy[57,58]) can be of the same order of magnitude as the host–guest binding energy. Thus, the calculated free energy of benzene binding to a *mutant of T4 lysozyme* in water varies between -7 and -9 kcal/mol, whereas the ''cratic'' free energy has been estimated to be $+7$ kcal/mol.[58] Upon complexation of the three guests by **L** considered in our study, the loss of rotational freedom may not be comparable. The free energy of rotation of the free guest calculated in the rigid rotor approximation with MacroModel varied between -5.6 (CH_2Cl_2) and -6.6 (CCl_4) kcal/mol. These values correspond to the ''upper'' limit of the cratic energy; that is, to a complete immobilization of the guest. In the **L · S** complexes simulated here, all guests rotated more or less inside the host's cavity. *A priori*, steric restrictions in this motion increased with the size of **S**. Thus, for the **L**·$CHCl_3$ complex, an increase of R^*_{Cl} from 1.947 to 2.14 Å slowed down the exchange between forms I and III, whose lifetime increased from 20–60 ps to 100–200 ps (Fig. 2).

Conclusions

Molecular dynamics and free-energy simulations have been performed on the macrocyclic ligand *cryptophane-E* and its complexes with neutral tetrahedral guests (**S** = CH_2Cl_2, $CHCl_3$, and CCl_4) in the gas phase and in chloroform solution.

The host cavity is relatively rigid and keeps a nearly constant size with the three tetrahedral guests. In the uncomplexed state, this cavity tends to be filled by bridging propylene and methoxy fragments in the gas phase. In chloroform solution, it contains one complexed solvent molecule.

Our study shows that the van der Waals parameters for the Cl atoms critically determine the complexation selectivity of **L**. Thus, calculations with Cl parameters of the guests taken from OPLS chloroform lead to preference of **L** for CCl_4 over $CHCl_3$, in contrast to complexation experiments in tetrachloroethane solution. Free energies of mutations **L**·$CHCl_3$ → **L·S** (**S** = CH_2Cl_2, CCl_4) were calculated systematically as a function of the R^*_{Cl} and ε_{Cl} parameters, with two different sets of charges. A good fit with experimental relative affinities can be obtained using the Lennard–Jones 6-12 parameters, with standard combination rules for unlike atoms, with electrostatic charges and the vdW parameters of $R^*_{Cl} = 2.14$ Å, $\varepsilon_{Cl} = 0.30$ kcal/mol. *Although the complexes are of the van der Waals type, it has been shown that their electrostatic representation is also important for correct calculation of the relative binding affinities*.

Finally, solvent effects play an important role in complexation selectivity; relative free energies of desolvation are of the same order of magnitude as relative intrinsic energies.

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